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Phomopsis longicolla Hobbs UZROČNIK TRULEŽI SJEMENA SOJE U HRVATSKOJ

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SAŽETAK

Soja (Glycine max (L.) Merrill), jedna je od najstarijih i najvažnijih kulturnih biljaka u svijetu. Na našim prostorima soja se kontinuirano sije od 1970. godine, a o značajnijoj proizvodnji (na oko 20 000 ha) može se govoriti krajem tek 80-ih godina prošloga stoljeća. U 2003. godini u Republici Hrvatskoj zasijano je 48 000 ha soje, s prosječnim urodom 2 708 kg/ha, što gotovo zadovoljava domaće potrebe za tom kulturom.

Jedan od osnovnih problema koji donosi povećanje površina i intenziviranje proizvodnje soje svakako je problem bolesti i to prije svega bolesti uzrokovanih fitopatogenim gljivama na koje otpada oko 80% svih bolesti soje, dok preostalih 20% uzrokuju bakterije i virusi. U oplemenjivačkom programu soje u Poljoprivrednom institutu Osijek, pored glavnih ciljeva stvaranja kultivara visokog i stabilnog uroda zrna visoke kakvoće, osobit značaj pridaje se zadovoljavajućoj otpornosti i tolerantnosti na značajne gljivične bolesti u našim uvjetima uzgoja. Nekoliko je bolesti koje se gotovo redovito javljaju na soji u našim agroekološkim uvjetima. To su prije svih plamenjača soje (Peronospora manshurica (Naum.) Syd. ex Gaum.) i bolesti koje uzrokuju gljive iz kompleksa Diaporthe/Phomopsis. Phomopsis longicolla Hobbs, (teleomorf nepoznat) se razlikuje od ostalih varijeteta iz kompleksa Diaporthe/Phomopsis po svojim karakteristikama u kulturi, morfologiji i po mjestu zaraze (prvenstveno uzrokuje trulež sjemena), i jedan je od najopasnijih patogena sjemena u glavnim područjima uzgoja soje u svijetu. S obzirom na to da je gljiva otkrivena i opisana u nama susjednim zemljama, a sumnja se na njenu prisutnost i kod nas, postoji opasnost da bi mogla postati značajan patogen te važne industrijske biljke i u našoj zemlji.

Cilj istraživanja bio je utvrditi prisutnost gljive pregledom sjemena soje iz široke proizvodnje s nekoliko lokaliteta i tri godine (2000., 2001. i 2002.), proučiti i opisati morfološke karakteristike patogena, determinirati patogena pomoću morfoloških karakteristika u kulturi i PCR-RFLP metode, utvrditi razlike u patogenosti izolata umjetnom infekcijom sjemena soje u laboratoriju te utvrditi razlike u reakciji genotipova soje na patogena inokulacijom sjemena soje pregledom sjemena nekoliko genotipova soje (promising lines) s pokusnih parcela lokaliteta Osijek u 4 godine (1999.-2002.) i postaviti poljski pokus nekoliko genotipova soje (promising lines) na lokalitetu Osijek, na kojemu je obaviti umjetnu infekciju najagresivnijim izolatom.

Izolacija gljive obavljena je iz sjemena soje sa simptomima koji odgovaraju onima koje izaziva Phomopsis longicolla Hobbs, a izdvojeno je iz uzoraka prikupljenih tijekom tri godine (2000., 2001. i 2002.) iz široke proizvodnje s lokaliteta: Nova Gradiška, Magadenovac, Valpovo, Širine, Đakovo, Bilje, Klisa, Bobota, Ovčara, Novi Berak, Tovarnik, Kneževo, Gorjani, Kutjevo, Valpovo.

Za proučavanje morfoloških karakteristika parazita korištena je metoda koju je opisao Hobbs i sur., (1985.).

Determinacija patogena obavljena je i pomoću biomolekularne metode lančanom reakcijom polimeraze (PCR-RFLP, Polymerase Chain Reaction – Restriction Fragment Length Polymorphism), kojom je potvrđena pripadnost odabranih izolata vrsti P. longicolla Hobbs. Modificiranom metodom ekstrakcije po Cenis, (1992.) izdvojena je DNA patogena, koja je zatim amplificirana i, naposljetku, digestijom pomoću restrikcijskih enzim, određena pripadnost izolata. Korištena je metoda koju su opisali Zhang et al., (1998.) i Riccioni et al., (1998., 2003.).

Proučavanje patogenosti izolata provedeno je inokulacijom sjemena soje u klima-komori i u posudama.

Nakon utvrđivanja najpatogenijeg među odabranim izolatima u ranije provedenim pokusima, ponovljeni su pokusi umjetne infekcije sjemena (u klima-komori i u posudama), ali ovoga puta koristeći najpatogeniji izolat i nekoliko novostvorenih genotipova soje (promising lines).

Uzorci sjemena pet genotipova kreiranih na Poljoprivrednom institutu Osijek iz prethodne 4 godine (1999., 2000., 2001. i 2002.) s lokaliteta Osijek (eksperimentalno polje Poljoprivrednog instituta) analizirani su na prisutnost P. longicolla.

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Poljski pokus je zasijan s 5 genotipova soje Poljoprivrednog instituta Osijek (Tablica 3.), 0 - I grupe zriobe u 4 ponavljanja na lokalitetu Osijek. U svakom ponavljanju zasijana je kontrolna varijanta za svaki genotip, tako da broj parcela u pokusu iznosio 40 (5 x 2 x 4). Pokus je postavljen kao strip-plot design, a parcele su inokulirane prskanjem suspenzijom konidija u R_6 – R_7 fenofazi (Fehr i sur., 1971.).

Statistička obrada podataka obavljena je pomoću programa Statistical Analysis System Version 8.2 (SAS Institute) analizom varijance (ANOVA) i testom za utvrđivanje značajnosti razlika (Least Significant Difference).

Morfološke karakteristike izolata proučavanih u ovom radu odgovaraju karakteristikama P. longicolla kako su opisali Hobbs i sur., (1985.) te pokazuju da je taj patogen prisutan i na soji u Hrvatskoj. Svi naši izolati podvrgnuti su analizi pomoću navedenih metoda ekstrakcije, amplifikacije i digestije rDNA i prema ovome svi naši izolati pripadaju gljivi Phomopsis longicolla Hobbs.

Uspoređujući sve pokazatelje pokusa u klima komori, uočava se da je umjetna infekcija izolatom Pl 027, najviše utjecala na broj trulih zrna, na smanjenje isklijalih zrna, značajno utjecala na dužinu klice i najviše od svih izolata utjecala na nekrozu klice. Zbog toga je izolat Pl 027 izabran kao najpatogeniji za proučavanje razlika u osjetljivosti/otpornosti genotipova u uvjetima umjetne infekcije. S obzirom na to da se i u pokusu u posudama izolat Pl 027 pokazao kao jedan od najpatogenijih (zajedno sa Pl 041), isti je upotrebljen za inokulaciju različitih genotipova soje u cilju utvrđivanja mogućih razlika u osjetljivosti/otpornosti na patogena P. longicolla.

Među genotipovima umjetno zaraženim gljivom P. longicolla u klima-komori nema potpuno otpornih, mada postoje značajne razlike u stupnju osjetljivosti. Rezultate bi svakako trebalo provjeriti u polju s obzirom na veliki utjecaj okolinskih činitelja, kako na patogena, tako i na svaki pojedini genotip. U pokusu u posudama, promatrajući inokuliranu varijantu, genotip OS-8 (2,75 biljaka po ponavljanju tijekom cijelog pokusa), bio je najosjetljiviji u usporedbi s ostalim proučavanim genotipovima, dok je genotip OS-139 imao značajno veći broj preživjelih biljaka (7,50 po ponavljanju). U kontrolnoj varijanti, svi genotipovi imali su zadovoljavajuću klijavost i nisu se međusobno značajno razlikovali.

U uvjetima prirodne infekcije, u prve tri godine (1999., 2000., i 2001.) niti na jednom od proučavanih genotipova soje nije utvrđena zaraza gljivama iz kompleksa Diaporthe/Phomopsis. U posljednjoj godini (2002.), kod svih genotipova, zabilježeno je prisustvo patogena iz tog kompleksa. Zaraza se kretala od 6% kod genotipa OS-101, zatim 13% (OS-109), 15% kod genotipovaOS-8 i OS-49 do 16% kod genotipa OS-139. D. phaseolorum var. caulivora bio je najčešće izolirani patogen Diaporthe/Phomopsis kompleksa iz sjemena soje i činio je 60 % svih nalaza. Udio gljive D. phaseolorum var. sojae iznosio je 21,54%, dok je za gljivu P. longicolla zabilježeno 18,46% zaraženih zrna. U pripremi sjemena soje za sjetvu nije obaveza obaviti specifične analize kao što je otkrivanje patogena Diaporthe/Phomopsis na sjemenu. Držimo, na temelju naših saznanja i podataka u literaturi, da bi ovome trebalo posvetiti veću pozornost i to jednako u oplemenjivačkim programima i sjemenarenju.

Na inokuliranim i na kontrolnim varijantama poljskog pokusa utvrđen je visok postotak sjemena soje zaraženog patogenima iz Diaporthe/Phomopsis kompleksa, iako je umjetna infekcija obavljena samo s P. longicolla. Očito je bilo infektivnog materijala svih patogena, a okolinski činitelji pogodovali su razvoju patogena. P. longicolla, unatoč umjetnoj infekciji, nije utvrđen na inficiranim genotipovima OS-8 i OS 139. Potpuno identični rezultati za te genotipove dobiveni su na kontrolnim parcelama. Uspješnost, odnosno neuspješnost, inokulacije, može se tumačiti, prije svega, povoljnim uvjetima za razvoj patogena. Diaporthe phaseolorum varijetet caulivora znatno je patogeniji od ostalih gljiva iz tog kompleksa. Prevladavanje jedne patogene vrste u povoljnim okolinskim uvjetima i u prisutnosti odgovarajućeg domaćina vrlo je složeno. Moguće je da se radi o kompeticijsko-konkurentskim odnosima između srodnih ili biološki udaljenih vrsta. Također, moguće je u pitanju inducirana otpornost, odnosno da domaćin nakon infekcije jednim patogenom ne može naknadno biti zaražen drugim koji mu je srodan. To su pitanja koja čekaju odgovor u nekim drugim istraživanjima.

Ključne riječi: Phomopsis longicolla Hobbs, soja (Glycine max (L.) Merrill), sjeme, genotipovi, otpornost/tolerantnost

Phomopsis longicolla Hobbs CASUAL AGENT OF SOYBEAN SEED DECAY IN CROATIA

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Doctoral thesis [(2)]

SUMMARY

Soybean (Glycine max (L.) Merrill), is one of the oldest and most important cultural plants in the world. On our territory, soybean was sawn in continuity until 1970, but since the end of 80-s of the last century it could be considered as a significant production (about 20 000 ha). In 2003 48 000 ha of soybean, with average grain yield 2 708 kg/ha, that almost meet domestic needs for this crop were sown in The Republic of Croatia.

One of the basic problems which occurs with increasing of acreages and more intensive soybean production is a disease problem. First of all diseases were caused by phytopathogen funguses, causing about 80% of all soybean disease, and rest of 20% were caused by bacteria and viruses. In soybean breeding program at the Agricultural Institute Osijek, besides production aiming at high and stabile yielded cultivars with high grain quality, special importance is given to satisfactory resistance and tolerance of significant fungal diseases in our growing conditions.

There are some diseases which almost regularly appear on soybean in our agroecological conditions. First to mention is downy mildew (Peronospora manshurica (Naum.) Syd. ex Gaum.) and diseases caused by fungus from Diaporthe/Phomopsis Complex. Phomopsis longicolla Hobbs, (teleomorf unknown). Above mentioned is different among other varieties, for its his cultural characteristic, morphology and place of infection (primary causing seed decay), and one of the most dangerous seed pathogens in main areas of soybean production in the world. Considering that fungus was discovered in our neighbouring countries there is suspicion of its presence in Croatia, danger of becoming a significant pathogen of this important industrial plant stands as a danger for our country as well.

The aim of investigation was to establish presence of fungus by seed examination on several locations from a large-scale production and three year (2000–2002) studying of morphological characteristics of the pathogen, determine pathogen by culture morphological characteristics and by PCR-RFLP method, establish differences in isolate pathogenicy by artificial seed infections in the laboratory, establish differences in soybean genotype reaction on pathogen by examining seed of some soybean cultivars (promising lines) from Osijek experimental field in four years (1999-2002),set a field trial of some genotypes on Osijek location and to carry out an artificial infection with the most aggressive isolate.

Fungus isolation was accomplished from soybean seed with symptoms corresponding to the one caused by Phomopsis longicolla Hobbs, and separated from samples being collected during three years (2000-2002) from large-scale production on the locations as follows: Nova Gradiška, Magadenovac, Valpovo, Širine, Đakovo, Bilje, Klisa, Bobota, Ovčara, Novi Berak, Tovarnik, Kneževo, Gorjani, Kutjevo, Valpovo.

Method described by Hobbs et al., (1985) was used for the investigation of pathogen morphological characteristics.

Determination of pathogen was carried out by a biomolecular method PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism), used to confirm a belonging of selected isolates to species P. longicolla Hobbs. By Cenis (1992) modified method of extraction DNA pathogen was extracted and then amplified, and finally isolate belongings were determined with restriction enzymes. Method described by Zhang et al., (1998) and Riccioni et al., (1998, 2003) was used.

Investigation of an isolate pathogenicy was carried out by soybean seed inoculation in wet chamber and pots. After establishing most of the pathogen among selected isolates in early conducted trials, those with artificial seed infection were repeated (in wet chamber and pots), but this time using the most pathogen isolate and several newly created soybean genotypes (promising lines).

Seed samples of five genotypes 2002 from Institutes' experimental field created in The Agricultural Institute Osijek in the period 1999 were analyzed for presence of P. longicolla.

Field trial was conducted with five soybean genotypes, 0-I maturity group in four replications on the Osijek location. Control of each genotype was sown for each replication, so total number of pots in trial was 40 (5 x 2 x 4). Trial was set as a strip-plot design, and lots were inoculated by spraying with conidial suspension in R_6 – R_7 stage of development (Fehr et al., 1971).

Statistical data processing was carried out by computer software Statistical Analysis System Version 8.2 (SAS Institute) with analysis of variance (ANOVA) and LSD (Least Significant Difference) test.

Morphological characteristics of isolates investigated in this work correspond to characteristics of P. longicolla described by Hobbs et al., (1985.) and show that this pathogen is present on soybean in Croatia. All our isolates were analyzed by mentioned methods of extraction, amplification and rDNA digestion, and according to this, all of them belong to fungus Phomopsis longicolla Hobbs.

Comparing all indicators from wet chamber trial, it could be seen that artificial infection with isolate Pl 027 have had the strongest influence on the number of rotten seed, on decreasing number of germinated seeds, significantly influenced on germ length, and among all isolates have had the strongest influence on germ necrosis. For all these reasons, isolate Pl 027 was selected as the most pathogen for investigation differences in genotype tolerance/resistance in artificial infection conditions. Since the isolate Pl 027 had been shown as one of the most pathogen in pot trials (together with Pl 041), the same one was used for inoculation of different soybean genotypes in order to determine possible differences in tolerance/resistance of pathogen P. longicolla.

Among genotypes being artificially infected with fungus P. longicolla in wet chamber, no one is totally resistant, although there are significant differences in susceptibility level. Results should be checked in the field due to great impact of environmental factors on pathogen as well as on each genotype. In pot trial, observing inoculated treatment, genotype OS-8 (2,75 plants per replication during all trials) was the most susceptible in comparison with other investigated genotypes, while genotype OS-139 had significantly higher number of survived plants (7,50 per replication). Control has shown, however, that all genotypes had satisfactory germination and there were no significant differences among them.

In first three years (1999-2001) in natural infection, no investigated soybean genotypes weren't established infection caused by Diaporthe/Phomopsis Complex fungus. In 2002 presence of pathogens from this complex were recorded for all genotypes. Infection varied from 6% for genotype OS-101, 13% (OS-109), 15% for genotypes OS-8 and OS-49 to 16% for genotype OS-139. D. phaseolorum var. caulivora was the most isolated pathogen of Diaporthe/Phomopsis Complex from soybean seed and made 60 % of all findings. Share of fungus D. phaseolorum var. sojae was 21,54% and for fungus P. longicolla was 18,46% infected seeds. In soybean seed preparation for sowing it is not obliged to carry out specific analysis like discovered pathogens from Diaporthe/Phomopsis Complex on seed. Therefore, based on our findings and data from literature we think that more attention should be paid on this issue in breeding programs as well as in seed production.

High percentage of soybean seeds diseased with pathogen from Diaporthe/Phomopsis Complex was established on inoculated and control plots of field trials, although artificial infection was carried out only with P. longicolla. Obviously infective material of all pathogens was present, and environmental conditions were suitable for pathogen development. In spite of artificial infection P. longicolla, wasn't established on inoculated genotypes OS-8 and OS 139. Completely identical results for these genotypes were also obtained on the control plots. Successful, or unsuccessful artificial inoculation, could be explained above all with suitable environmental factors for pathogen development. Diaporthe phaseolorum variety caulivora is significantly more pathogen than other fungus of this complex. Prevalence of one pathogen in favorable environmental conditions and presence of suitable host is very complex. It is possible that this is competitive relationship among related or biologically distant species. Also, it is possible that "so called" induced resistance is present, which means that the host after infection with one pathogen couldn't be infected later with other related. These are questions waiting for answers in some other investigations.

Key-words: Phomopsis longicolla Hobbs, soybean (Glycine max (L.) Merrill), seed, genotypes, resistance/tolerance

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